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Construction of a probe-immobilized molecularly imprinted electrochemical sensor with dual signal amplification of thiol graphene and gold nanoparticles for selective detection of tebuconazole in vegetable and fruit samples

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ABSTRACT

A probe-immobilized molecularly imprinted electrochemical sensor was constructed for selective determination of tebuconazole in vegetable and fruit samples. Thiol graphene was introduced onto a glassy carbon electrode modified with gold nanoparticles to increase its specific surface area. Prussian blue was co-deposited with gold nanoparticles on the modified electrode and served as the immobilized probe, followed by electro-polymerization of the molecularly imprinted polymer film as the recognition element. Systematic validation and characterization were performed to verify the successful preparation and mechanisms of the Prussian blue and molecularly imprinted polymers in the electrode. Several important parameters, including monomer concentration, scan cycles and pH, were systematically varied to elucidate their influence on the performance of the sensor. Meanwhile, the surface features of the modified electrode were characterized using scanning electron microscopy, atomic force microscopy, cyclic voltammetry and electrochemical impedance spectroscopy. The optimized sensor demonstrated a wide linear range from 5.0×10^{-8} mol L^{-1} to 4.0×10^{-4} mol L^{-1} with a detection limit of 1.25×10^{-8} mol L^{-1}. The proposed sensor exhibited specific recognition of tebuconazole in selectivity experiments and contrast tests. Furthermore, the sensor can be used for detection of tebuconazole in real samples with satisfactory recoveries.

1. Introduction

Tebuconazole, a triazole fungicide, is widely used in agricultural production for controlling pathogenic fungi on vegetables, fruits and grains to ensure the yield and quality of agricultural products. However, frequent use and abuse of the fungicide has deleterious effects, such as potential harm to human health and environmental pollution resulting from residue in agricultural products, soil and water [1]. Hence, detection technologies are required to monitor the pesticide residue in food and the environment. A large number of highly sensitive methods, such as surface plasmon resonance (SPR) spectroscopy [2], gas chromatography (GC) [3], and high-performance liquid chromatography (HPLC) [4,5], have been developed for tebuconazole residue analysis in various matrices. Accurate qualitative and quantitative results can be achieved by chromatographic methods. However, the disadvantages of expensive instruments, lengthy steps and high skill requirements make it difficult to achieve rapid on-site detection of tebuconazole in real

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samples. Therefore, it is important to develop a sensitive and rapid method for the detection of tebuconazole. Electrochemical technology has the advantages of low cost, a convenient process and high sensitivity [6]. Unfortunately, there are few reports on the electrochemical detection of tebuconazole in real samples as a result of its lack of electro-activity.

For compounds without electro-activity, selection of a recognition element and an indicator molecule are the keys to obtaining a sensitive and selective method. Recently, molecularly imprinted polymer (MIP) has attracted broad attention due to its selectivity and recognition capability for target compounds coupled with electrochemical technology [7,8]. MIP film is fabricated and based on copolymerization of functional monomers in the presence of template compounds, which results in a three-dimensional substrate with specific recognition capability [9]. Subsequent removal of the template molecule from the polymer matrix generates nanocavities, which are in accordance with the shape, size and functional groups of the template. The MIP film with specific recognition for the template compound is thus obtained [10]. However, achieving sufficient sensitivity is a significant challenge due to the difficulty of completely extracting the deeply embedded template molecules from the polymer matrix. Here, nanomaterials can play an important role to enhance sensitivity. Nanomaterials, including nanoparticles, nanotubes and nanocomposites, have been used to increase the surface area and further improve the sensitivity of the modified electrode [11]. Both gold nanoparticles, graphene [12,13] and carbon nanotubes have proven to be effective in signal amplification and in improving surface area.

A further challenge is to identify and determine the concentration of tebuconazole after successful capture by the MIP sensor. Generally, an electrochemical redox mediator, such as haematein, is used as an indicator in the supporting electrolyte solution. It is worth noting that a redox mediator in the electrolyte would pollute the samples and influence the accuracy of the sensor. Therefore, the immobilization of a redox mediator on electrode is preferable to ensure the accuracy and convenience of detection. K_3 [Fe(CN)$_6$] and K_4 [Fe(CN)$_6$] solutions are widely used as detection probes. Hence, the idea arose to immobilize Prussian blue (PB, Fe_4 [Fe(CN)$_6$]$_3$) on the electrode. PB can be prepared by microwave heating [14], chemical solution [15,16], self-assembly [17] and catalytic growth [18,19]. Nevertheless, it is difficult to obtain uniform PB films directly on the electrode surface by the aforementioned methods. Alternatively, in-situ electro-polymerization has been reported for the preparation of a PB film directly on a Pt electrode for detection of oxytetracycline based on the catalysis of inorganic PB films and the enzymatic effect of glucose oxidase [20]. Therefore, electropolymerization was attempted to construct an in situ PB film, which acted as a detection probe for the direct determination of tebuconazole.

The present work describes the design and construction of a probe-immobilized molecularly imprinted electrochemical sensor to achieve sensitive, selective and rapid detection of tebuconazole. Gold nanoparticles (Au NPs) were first fixed onto an electrode as a coupling agent for further modification. Thiol graphene (SH-G) was introduced *via* the Au-S interaction to improve the specific surface area, followed by the electro-deposition of Au-PB onto the SH-G as an electrochemical indicator. The MIP film was fabricated and used as a recognition element to capture the target molecules. The preparation process of each film was systematically characterized to prove its successful immobilization. The performance of the resulting sensor was validated to demonstrate its feasibility in tebuconazole residue analysis in real samples.

2. Experimental

2.1. Chemicals and apparatus

Tebuconazole, triadimenol and bitertanol (purity ≥ 97.0%) were obtained from Dr. Ehrenstorfer (Ausburg, Germany). Penconazole (98.1%) and myclobutanil (99.2%) were purchased from Shanghai Pesticide Research Institute (Shanghai, China). Acetamiprid (98.2%) was purchased from Agro-environmental Protection Institute, Ministry of Agriculture (Tianjin, China). *O*-aminophenol and resorcinol were purchased from TCI Co., Ltd. (Shanghai, China). SH-G was purchased from TanFeng technology limited company of Suzhou (Suzhou, China). HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Acetic acid, H_2SO_4, Na_2HPO_4, NaH_2PO_4, K_3 [Fe(CN)$_6$], K_4 [Fe(CN)$_6$], HNO_3 and KCl were of analytical purity. H [$AuCl_4$] was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Ultrapure water was used for all experiments. Stock solutions of *o*-aminophenol and resorcinol (100 mmol L^{-1}) were prepared in methanol. A 0.05 mol L^{-1} phosphate buffer solution (PBS, pH 7.0) was prepared with Na_2HPO_4 and NaH_2PO_4 solutions.

Field-emission scanning electron microscopy (FE-SEM) was performed on an Utral 55 instrument (Corl-zeisD, Germany) for the morphologic characterization of the modified electrodes. The surface topography of the modified electrodes was observed by atomic force microscopy (AFM) on a Multimode Nanoscope IIIa Controllor (Veeco Instruments Ins., US). An Autolab PGSTAT 204 workstation (Metrohm, Switzerland) was used for the preparation of the modified sensor under three electrodes, with a bare glassy carbon electrode (GCE) or modified GCE as the working electrode, a saturated calomel electrode as the reference electrode and a platinum wire as the counter electrode. All the experiments were carried out at room temperature.

2.2. Preparation of the probe-immobilized MIP sensor

Before modification, the GCE was polished with 0.05 μm alumina slurries, followed by ultrasonic treatment in water for 5 min. The electrode was scanned by cyclic voltammetry (CV) between −0.4 V and 1.6 V at a scan rate of 100 mV s^{-1} in 0.1 mol L^{-1} H_2SO_4 solution for 20 cycles. The clean GCE was modified as the following procedures.

(1) Modification of Au NPs and SH-G. The GCE was immersed in a 3 mmol L^{-1} H [$AuCl_4$] solution and treated at a constant potential of −0.2 V for 100 s, yielding Au NPs on the GCE surface. SH-G (5.0 mg) and water (20 mL) were mixed and sonicated for 15 min to form a homogeneous solution. Subsequently, the Au NPs-modified GCE was immersed in the SH-G solution for 3 h to obtain the SH-G modified electrode, which is termed SH-G/Au NPs/GCE.
(2) Electro-deposition of Au-PB. The surface of SH-G/Au NPs/GCE was modified with Au-PB by performing CV from 0 to 1.0 V for 17 cycles in a solution containing KNO_3 (0.1 mol L^{-1}), K_3 [Fe(CN)$_6$] (1.0 mmol L^{-1}) and H [$AuCl_4$] (1.0 mmol L^{-1}). The modified electrode, termed Au-PB/SH-G/Au NPs/GCE, was thoroughly rinsed with water.
(3) Electro-polymerization of the MIP. The electropolymerization solution contained 2.1 mmol L^{-1} *o*-aminophenol, 2.1 mmol L^{-1} resorcinol and 0.7 mmol L^{-1} tebuconazole. After deoxygenating the solution by bubbling with high purity nitrogen for 10 min, the Au-PB/SH-G/Au NPs/GCE electrode was immersed in the electro-polymerization solution. The MIP membrane was fabricated by performing CV